THE IDENTITY OF *LEISHMANIA TROPICA* WRIGHT, 1903, AND *HERPETOMONAS PAPATASII* ADLER, 1925

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PLATES XXVIII, XXIX

The authors (1925 and 1926) have recorded three cases of experimental transmission of cutaneous Leishmaniasis to man from naturally infected specimens of *Phlebotomus papatasii* ♀♀. These experiments in themselves are not sufficient to prove that the sandfly is a natural carrier of cutaneous Leishmaniasis, for since Laveran and Franchini (1913 and 1916) infected mice with *Herpetomonas* from various insects, Fantham and Porter (1914 and 1915) infected animals with *Herpetomonas* from species as widely removed from each other as *Nepa cinerea* and *Ctenocephalus canis*, and Fantham (1922) produced Leishmaniasis in a young rat by injecting it with *Herpetomonas muscae domesticae*, it can be argued that any *Herpetomonas*, whatever its source, if introduced into the human skin, may give rise to cutaneous Leishmaniasis. It is true that Hoare (1921), Becker (1923), Shortt (1923) and Drbohlav (1925), among others, have repeated the experiments of Laveran and Franchini and of Fantham and Porter, with negative results but, as Becker has pointed out, these results 'can have such value only as is ordinarily attributable to negative results.'

There is one point of particular importance for the interpretation of negative results in experiments with *Herpetomonas* from insects on man or animals which has been overlooked by all writers on the subject, i.e., *Herpetomonas* sp. in insects are polymorphic and not

all the forms are necessarily infective. The Leishmania forms and many of the flagellate forms in artificially infected *P. papatasii* are not infective for man as the experiments of Adler and Theodor (1926) conclusively prove.*

Evidence is accumulating to the effect that the development of *Leishmania tropica* in *Phlebotomus papatasii* is a biological one with infective forms as the end point of the cycle in the sandfly as predicted by Adler (1925). Infective forms need not necessarily be present in an insect containing *Herpetomonas* and this may explain some of the negative results obtained in experiments with *Herpetomonas* on animals. For the same reason the *uniformly* positive results of Fantham and Porter are difficult to understand.

Noguchi (1924) and Kligler (1925 and 1926) independently introduced agglutination methods which demonstrate the specificity of the three species of *Leishmania* of man, methods which promise to simplify the whole problem of experimental Leishmaniasis and liberate it from the conflicting evidence so far obtained from the injections of *Herpetomonas* from various insects into animals. Agglutination methods promise to be of great value not only in Leishmaniasis but also in the study of cultivable *Herpetomonas* in general. Hitherto it has been customary to name every *Herpetomonas* found in a new host as a new species, for differentiation of species on morphological grounds is within wide limits obviously impossible.

Becker (1923) and Drbohlav (1925) have successfully carried out cross-infection experiments with *Herpetomonas* from various flies and on the basis of these cross-infection experiments Becker considers it extremely probable that *Herpetomonas muscae domesticae, H. luciliae, H. calliphorinae* and *H. sarcophagae* are one species and Drbohlav considers the Herpetomonas of *Lucilia serricata, L. cesarea, Fannia regina* and *Musca domestica* as identical. It must be pointed out that cross-infection experiments in insects and in general artificial inoculation of insects with *Herpetomonas,* though suggestive, can never in themselves be conclusive and may actually prove misleading,

* Sergent, Edm. and Et., Parrot, L., Donatien, H., and Béguet, M. (1926) have recently republished, with many additional details, their experiment of 1921 in which they produced oriental sore in man by inoculation of an infusion of seven sandflies. In the original paper (1921) they state, ' Le liquide de broyage, examiné au microscope, ne montre ni flagellés ni aucune autre forme parasitaire,' and they repeat this statement in their recent paper. As it is inconceivable that the result obtained should have been produced in the absence of *Herpetomonas* we are convinced that *Herpetomonas* were present but were overlooked.

e.g., the bed bug which is a natural host of neither *Herpetomonas donovani* nor *H. tropica* can be artificially infected with both these flagellates. On the other hand one species of flagellate may be inoculable into a various number of arthropod hosts, e.g., *Schizotrypanum cruzi* is infective for *Conorrhinus megistus, Cimex lectularius* and *Ornithodorus moubata.* It thus appears certain that the method of the future for determining *Herpetomonas* sp. will be a serological one. Such a method, if successful, will greatly facilitate investigations on oriental sore and Kala-azar for if an insect is suspected of being a carrier of these diseases, a comparison of its naturally occurring *Herpetomonas* with cultures of *Leishmania donovani* and *L. tropica* by Noguchi's or Kligler's method will quickly confirm or dispel this suspicion. Serological methods should also prove decisive in settling the long-standing problem of the relationship between human and canine Leishmaniasis in localities where these two diseases are prevalent.

The three experimental strains were compared by cross agglutinations with three strains from naturally acquired oriental sores.

EXPERIMENTAL STRAINS

Strain I. The lesion in this case was a papule. By April 26th the papule was 3 mm. in diameter, Leishman-Donovan bodies which had hitherto been few became numerous and cultures were readily obtained.

Strain II. The lesion consisted of a hard subcutaneous nodule.

Strain III. The lesion consisted of two ulcers.

The above lesions and the details of their transmission and development were described in a previous communication. In the case of Strain III the two lesions healed spontaneously by July, 1926, i.e., about eight months after inoculation.

Strain IV. This strain was isolated by Professor I. J. Kligler, from an oriental sore acquired in Baghdad.

Strain V. Isolated by Professor I. J. Kligler, from a case from Artuf (Palestine).

Strain VI. Isolated by Dr. R. Junovitch, of the Rothschild Hospital, from a case acquired in Jerusalem.

All the strains were cultured on the following modification of Noguchi's leptospira medium :

Agar, 1 part.
Locke's solution containing 0·2 % dextrose, 8 parts.
Fresh rabbits' serum, 1 part.

On the above medium growth of *L. tropica* is fairly rapid, a uniform thick growth being produced in the upper 4 mm. of the tube five to seven days after inoculation.

The above medium was also found to be very satisfactory for the culture of *Schizotrypanum cruzi*.

Noguchi (1924) showed that if his leptospira medium is made up with immune rabbit serum instead of normal serum, the flagellates, instead of growing in a uniform layer at the top of the medium, grow in clumps for a varying distance throughout the medium ; also, if immune serum is added to normal cultures, these cultures become agglutinated. This reaction Noguchi showed to be specific for each of the three human species. We employed both methods for comparing the various strains. Immune serum for each strain was prepared by four intravenous injections into rabbits of pure culture at four days' interval, the first injection being 0·5 c.cs. and the fourth 2·0 c.cs. ; each rabbit received a total of 5 c.cs. pure culture.

With Noguchi's method the results were quite decisive and the six strains were found to be identical.

The growth in media made up with immune serum consists either of globoid masses (Pl. XXVIII, figs. 2-7) scattered through the medium, or globoid masses together with irregular granular flakes of various size (Pl. XXVIII, figs. 2-7). Each globoid mass consists of a homogeneous mass of Leishman-Donovan bodies (Pl. XXIX, figs. 1-4) and may or may not contain at its periphery a number of forms with rudimentary flagella. The irregular flakes consist of masses of Leishman-Donovan bodies and flagellates in varying proportion (Pl. XXIX, figs. 5-6).

The specific serum produces a far profounder effect on the organisms than mere agglutination for if subcultures on medium made up with normal serum are made from cultures grown on medium made up with 10 per cent. specific serum, growth takes place not in the upper three or four millimetres, as in a normal culture,

but from the surface down to a depth of several centimetres (Pl. XXVIII, figs. 8-9), a phenomenon noted by Noguchi to occur in normal cultures of *Leishmania donovani*.

This reaction, which shows a change of oxygen requirement on the part of the organism, is not due to agglutinin for the amount of serum picked up in a single loopful of culture is not sufficient to alter a normal culture. This change in oxygen requirement persists in successive subcultures for two to five generations.

Kligler's method consists of agglutination in test-tubes with various dilutions of serum. Its chief drawback lies in the difficulty of interpretation due to the tendency of *Leishmania tropica* in culture to form rosettes, and in the time required to make a suspension of *Herpetomonas* free from media. The following technique was used. One part pure culture is mixed with six parts saline and centrifuged ; the supernatant fluid is removed and replaced by an equal quantity of saline, the mixture is shaken and again centrifuged. This is repeated at least three times and finally a suspension is made of one part original pure culture to six parts saline. (The above method has been used by one of us (A.) during the last six months for the preparation of vaccine.) The suspension is divided into tubes and the agglutination is performed in the usual way. It is absolutely essential that the cultures used for the agglutination should not be recently isolated but should be at least five or six generations old, otherwise the suspension tends to contain granular masses of parasites and is useless for agglutination. Using the above method an immune serum gives a titre of up to 500.

The tubes are read after twenty-four hours. The following titres were obtained :

Serum 1, 100.
Serum 2, 200.
Serum 3, 500.
Serum 4, 200.
Serum 5, 100.
Serum 6, 200.

After reading, the tubes should be shaken and it will be noted that in the positive tubes a uniform suspension cannot be made, for granular masses of various sizes are distributed through the suspension. These masses on microscopic examination are found to be clumps of agglutinated organisms. If the control tubes contain

a deposit, which sometimes happens in spite of all care, a uniform suspension is formed on shaking. In lower dilutions (1 to 40) there is no difficulty in reading the results.

The above method cannot be considered final and much work still requires to be done in producing a technique of agglutination in tubes for *Herpetomonas* which will lend itself to standardisation.

It is interesting to note that Napier's formaldehyde test was negative in the three experimental cases and in the six immunised rabbits. During 1924 and 1925 one of us (A.) examined the sera of thirteen naturally infected cases of oriental sore from Dr. A. Dostrowsky's clinic for this reaction, and all were negative.

The serological tests prove that the parasite of all the three experimental lesions produced by the inoculation of *Herpetomonas* from naturally infected *Phlebotomus papatasii* ♀ into man was no other than *Leishmania tropica*, in other words, *Herpetomonas papatasii* is a synonym of *Leishmania tropica* and *Leishmania tropica* is a natural parasite of *Phlebotomus papatasii*.

The number of clinical varieties of oriental sore has given rise to the suspicion that there are varieties of *Leishmania tropica* and Brumpt has gone so far as to create a species *Leishmania nilotica* Brumpt, 1913, for the parasite of a nodular form of cutaneous Leishmaniasis occurring in the Sudan. The above serological observations show that three experimental lesions which clinically differed so markedly were caused by the same parasite and the correctness of the serological interpretation is proved by the following experiments.

Experiment I, 20.4.26. Volunteer inoculated (intracutaneously) on two points on left forearm direct from Case II (Nodule). 16.6.26. A papule was noted on one of the inoculated points and, on examination, Leishman-Donovan bodies were found.

Experiment II, 20.4.26. A volunteer was inoculated into two points on the left forearm direct from Case II. The skin was incised and the inoculation was made with a capillary into the subcutaneous tissue. No lesion has yet appeared (after four months).

The result of Experiment No. I proves that there is no relationship between a strain of *Leishmania tropica* and the clinical type of lesion produced in man which therefore depends entirely on factors present in the infected individual. We have to thank Professor I. J. Kligler and Dr. R. Junovitch for the gift of strains of *Leishmania tropica*.

CONCLUSIONS

Herpetomonas papatasii is a synonym of *Leishmania tropica*. *Leishmania tropica* is a natural parasite of *Phlebotomus papatasii* and the proof that the latter is a natural carrier of cutaneous Leishmaniasis is complete.

There is no relationship between a strain of *Leishmania tropica* and the clinical type of cutaneous Leishmaniasis produced in man.

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PLATE XXVIII

Fig. 1. Normal culture.

Figs. 2-7. Show types of agglutination in culture on media made up with immune serum. Note in each tube individual globoid masses and diffuse growth in varying proportions.

Fig. 2. Strain I grown on serum 2.

Fig. 3. Strain II grown on serum 2.

Fig. 4. Strain IV grown on serum 1.

Fig. 5. Strain III grown on serum 4.

Fig. 6. Strain IV grown on serum 2.

Fig. 7. Strain V grown on serum 4.

Figs. 8-9. Subcultures on media made up with normal serum from strains grown on immune serum. Note deep growth.

Fig. 8. Strain V.

Fig. 9. Strain II.

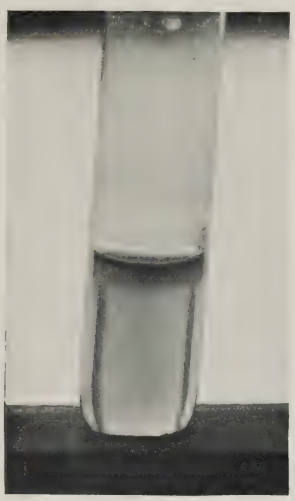

FIG. 1

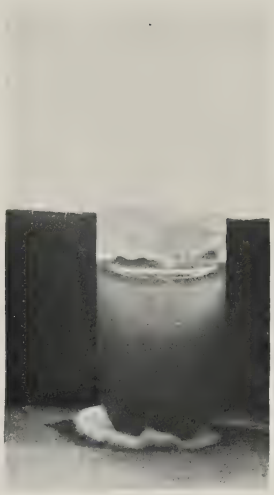

FIG. 2

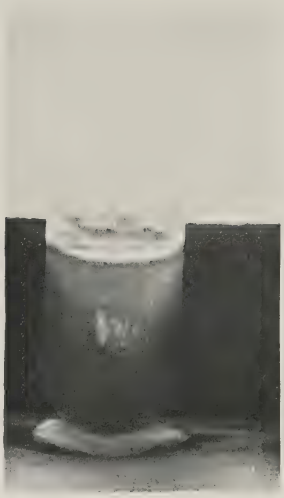

FIG. 3

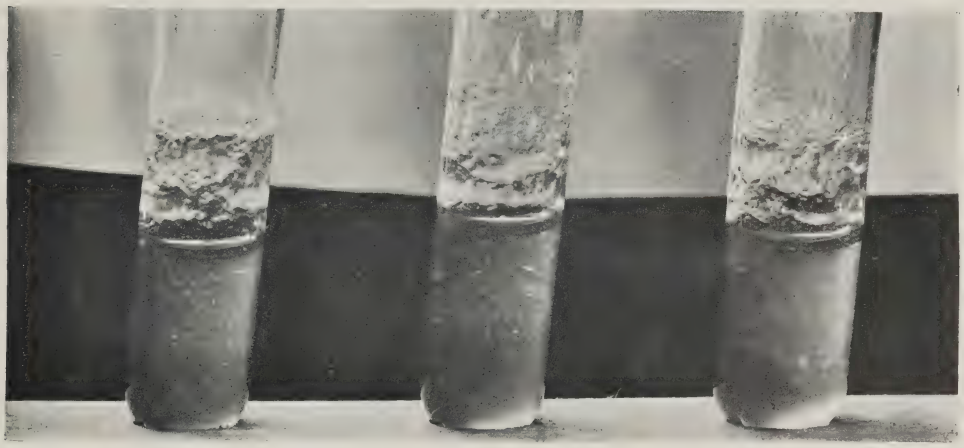

FIG. 4 FIG. 5 FIG. 6

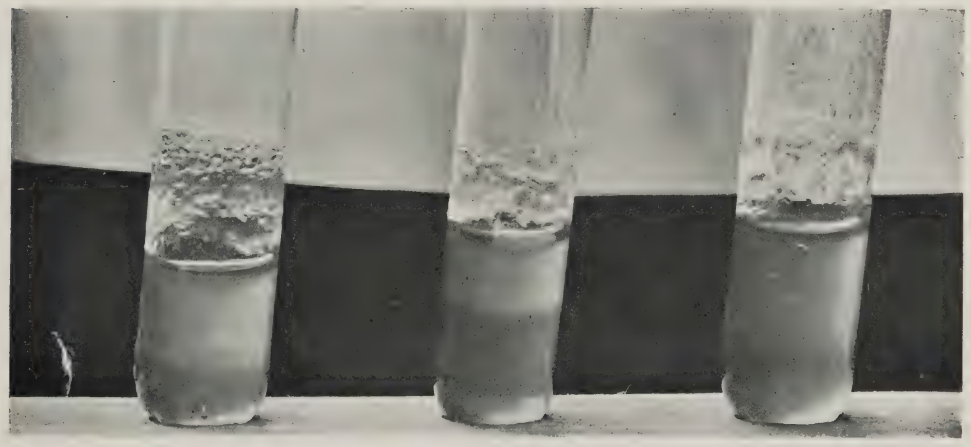

FIG. 7 FIG. 8 FIG. 9

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PLATE XXIX

Figs. 1-2. Colonies consisting entirely of Leishman-Donovan bodies from culture made up with immune serum. × 65.

Figs. 3-4. Same type of colonies. × 250.

Figs. 5-6. Masses consisting of Leishman-Donovan bodies and flagellates from diffuse growth in immune serum. × 250.

Figs. 7-8. Fields from a normal culture. Fig. 7. × 250. Fig. 8. × 200.

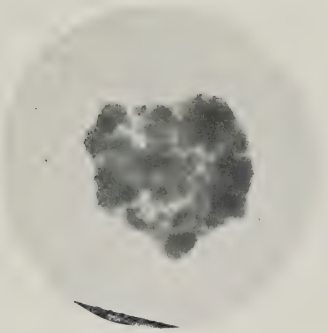


FIG. 1

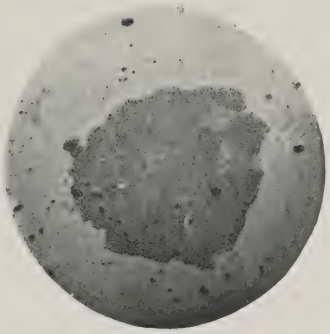


FIG. 2

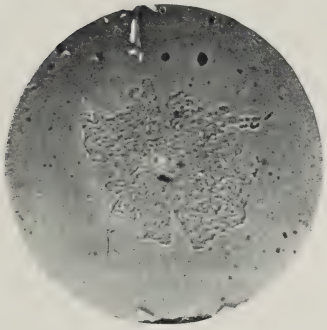


FIG. 3

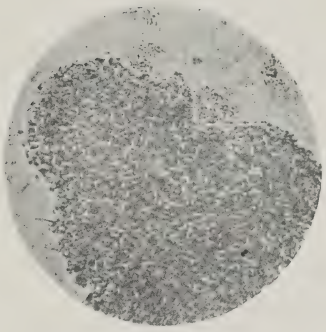


FIG. 4

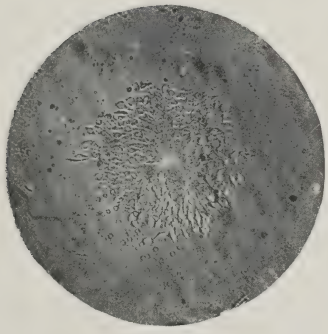


FIG. 5

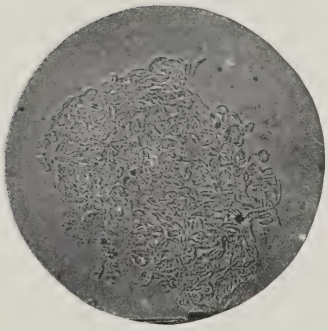
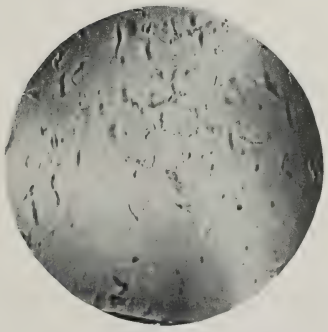


FIG. 6



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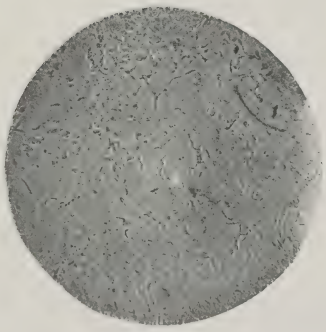

FIG. 8

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